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Ambient Water Quality Criteria for Benzidine

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16. ABSTRACT

Section 304(A) of the Clean Water Act [33 U.S.C. 1314(A)], requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for Benzidine. It presents concentration criteria for the protection of freshwater and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A Section 304(A) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality standard and in itself has no regulatory effect.

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AMBIENT WATER QUALITY CRITERIA FOR

BENZIDINE

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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Aquatic Life Toxicology

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CRITERIA DOCUMENT

BENZIDINE

CRITERIA

Aquatic Life

The available data for benzidine indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 2,500 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of benzidine to sensitive freshwater aquatic life.

No saltwater organisms have been tested with benzidine and no statement can be made concerning acute and chronic toxicity.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of benzidine through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 1.2 ng/l, 0.12 ng/l, and 0.01 ng/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 5.3 ng/l, 0.53 ng/l, and 0.05 ng/l, respectively.

INTRODUCTION

Benzidine (4,4'-diaminobiphenyl) is an aromatic amine. A proven human carcinogen, its primary site of tumor induction is the urinary bladder. It is also mutagenic.

The incidence of bladder tumors in humans resulting from occupational exposure to aromatic amines (benzidine) was first researched in Germany in 1895. The first cases of this condition in the United States were diagnosed in 1931 and reported in 1934.

Several studies implicating the high risk of bladder tumors in workers exposed to benzidine and other aromatic amines are well documented.

Adversary proceedings under section 307(a) of the Federal Water Pollution Control Act resulted in the promulgation of a toxic pollutant effluent standard for benzidine. The ambient water criterion upon which the standard was based was 0.1 µg/l (42 FR 2617).

Benzidine is an aromatic amine with a molecular weight of 184.24 (Weast, 1972). Existing as a grayish-yellow, white, or reddish-gray crystalline powder (melting point, 128°C; boiling point, 400°C) (Standen, 1972), benzidine's solubility increases as water temperature rises. The solubility of benzidine in 12°C water is 400 mg/l (Verschueren, 1977). Solubility is greatly enhanced with dissolution into organic solvents (Stecher, 1968). Its log octanol/water partition coefficient is 1.81 (Radding, et al. 1977). Benzidine is easily converted to and from its salt (Morrison and Boyd, 1972).

Diazotization reactions involving benzidine will result in colored compounds (color will vary with molecular structure). Because of their color, azo compounds are important as dyes for industrial use (Morrison and Boyd, 1972). The pKa values for the amino groups in benzidine were reported to be 3.57 and 4.66 (Weast, 1972).

Oxidation by metal cations appears to be an important route for benzidine degradation in the aquatic environment (Lahav and Raziel, 1971). Benzidine is not bioaccumulated to a significant extent by aquatic organisms, and it is apparently not easily degraded by the microorganisms in sewage plant sludge (Howard and Saxena, 1976).

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Aquatic Life Toxicology*

INTRODUCTION

Static, acute tests have been conducted with five freshwater fish species and a scud and bioconcentration factors range from 38 to 2,620. No data are available for saltwater species.

EFFECTS

Acute Toxicity

The 96-hour LC_{50} values for rainbow and lake trout, red shiner, and flagfish range from 2,500 to 16,200 µg/l (Table 1).

Comparable tests conducted with the fathead minnow and a scud, Gammarus pseudolimnaeus, resulted in no observed mortality at the highest test concentration of 20,000 µg/l (Table 3).

Residues

The bluegill has been exposed to ^{14}C-benzidine under flow-through conditions for 42 days (EG and G Bionomics, 1975). The edible portion of the fish bioconcentrated ^{14}C-residues by 38 to 44 times (Table 2). The comparable bioconcentration factor for the non-edible portion (viscera) of the bluegill ranged from about 12 to about 43 times that amount present in the edible portion. The half-life of ^{14}C-residues in the fish was about 7 days.

Miscellaneous

Lu, et al. (1977) studied the behavior of benzidine in a model ecosystem for 3 days and observed bioconcentration factors in algae, snails, mosqui-

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are the calculations for deriving various measures of toxicity as described in the Guidelines.

tos, and the mosquitofish of between 55 to 2,620 times. The result with the mosquitofish (55x) is consistent with that discussed earlier with the bluegill.

Summary

One freshwater invertebrate and five fish species have been exposed to benzidine under static acute test conditions. The 96-hour LC_{50} values for four fish species ranged from 2,500 to 16,200 µg/l. The LC_{50} values for the other species were greater than 20,000 µg/l. Bioconcentration factors for the bluegill ranged from 38 to 44 and, in a model ecosystem after 3 days, bioconcentration factors ranged from 55 for fish to 2,620 for an alga.

No saltwater organism has been tested with benzidine.

CRITERIA

The available data for benzidine indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 2,500 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of benzidine to sensitive freshwater aquatic life.

No saltwater organisms have been tested with benzidine and no statement can be made concerning acute or chronic toxicity.

Table 1. Acute values for benzidine (U.S. EPA, 1980)

Species	Method*	LC50/EC50 (µg/l)	Species Acute Value (µg/l)
	FRESHWATER SPECIES		
Rainbow trout, *Salmo gairdneri*	S, U	7,400	7,400
Lake trout, *Salvelinus namaycush*	S, U	4,350	4,350
Red shiner, *Notropis lutrensis*	S, U	2,500	2,500
Flagfish, *Jordanella floridae*	S, U	16,200	16,200

* S = static, U = unmeasured

No Final Acute Value is calculable since the minimum data base requirements are not met.

Table 2. Residues for benzidine (EG & G Bionomics, 1975)

Species	Tissue	Bioconcentration Factor	Duration (days)
	FRESHWATER SPECIES		
Bluegill, *Lepomis macrochirus*	edible portion	38 to 44*	42

* Results based on ^{14}C-residue content.

Table 3. Other data for benzidine

Species	Duration	Effect	Result (µg/l)	Reference
		FRESHWATER SPECIES		
Alga, *Oedogonium cardiacum*	3 days	Model ecosystem, bioconcentration factor = 2,620	-	Lu, et al. 1977
Snail, *Physa* sp	3 days	Bioconcentration factor = 645	-	Lu, et al. 1977
Scud, *Gammarus pseudolimnaeus*	96 hrs	LC50	>20,000	U.S. EPA, 1980
Mosquito (larva), *Culex pipiens*	3 days	Model ecosystem bioconcentration factor = 456	-	Lu, et al. 1977
Fathead minnow, *Pimephales promelas*	96 hrs	LC50	>20,000	U.S. EPA, 1980
Mosquitofish, *Gambusia affinis*	3 days	Model ecosystem, bioconcentration factor = 55	-	Lu, et al. 1977

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Mammalian Toxicology and Human Health Effects

INTRODUCTION

In general, exposure to benzidine compounds occurs in factories that synthesize benzidine and its congeners and convert them to dyes. It is also probable that some exposure occurs when the closed system used in synthesis is cleaned (Haley, 1975). Exposure also occurs from breathing contaminated air, ingesting contaminated food, and wearing contaminated clothing (Meigs, et al. 1951). Pointing of brushes by Japanese kimono painters results in the ingestion of benzidine dyes (Yoshida and Miyakawa, 1973), although ingestion is not generally an important source of exposure.

EXPOSURE

Ingestion from Water

Water could be contaminated with benzidine and its derivatives and dyes if plant water is discharged into water supplies serving a residential community. However, as of this time no reports of such contamination have appeared in the literature.

Ingestion from Food

While it is possible for food to become contaminated with benzidine and its derivatives under poor industrial hygienic conditions, ingestion of contaminated food is not a real contributor to the overall problem of benzidine toxicity.

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic animals seem to be proportional to the percent lipid in the tissue. Thus the per capita

ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States was analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

A measured steady-state BCF of 41 was obtained for benzidine using the edible portion of bluegills (EG & G Bionomics, 1975). A higher BCF was found for the nonedible portion, but percent lipids were not reported for either portion and the relative weights of the two portions were not reported. However, for 3,3'-dichlorobenzidine the BCF for whole body was about 3.5 times that for edible flesh (Appleton and Sikka, 1980). Thus, the steady-state bioconcentration factor for benzidine in the whole body of the tested bluegills can be estimated to be 140. Similar bluegills contained an average of 4.8 percent lipids (Johnson, 1980). An adjustment factor of 3.0/4.8 = 0.625 can be used to adjust the measured BCF from the 4.8 percent lipids of the bluegill to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for

benzidine and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 140 x 0.625 = 87.5.

Inhalation

In the early phases of the chemical and dye industries, the lack of good industrial hygienic practices and the use of open systems made inhalation one of the principal routes of entry of benzidine and its derivatives into the body. Similar inhalation exposures can occur at the present time unless workers wear respirators and protective clothing while cleaning the equipment (Haley, 1975).

Dermal

Skin absorption is the most important path of entry into the body. Intact skin is readily penetrated by benzidine and 3,3'-dimethylbenzidine (Meigs, et al. 1951). 3,3'-Dichlorobenzidine, because of its nonvolatility and large particle size, presents less of an inhalation and skin penetration hazard than benzidine (Gerarde and Gerarde, 1974; Rye, et al. 1970). It is the light, fluffy, powdery nature of benzidine base that poses the tumorigenic hazard to benzidine workers from skin absorption (Barsotti and Vigliani, 1952). The ease of skin penetration determines the following order of decreasing toxicity from these chemicals: benzidine, 3,3'-dimethoxybenzidine, and 3,3'-dichlorobenzidine (Rye, et al. 1970).

Environmental conditions of high air temperature and humidity increase skin absorption of benzidine, 3,3'-dimethoxybenzidine, 3,3'-dichlorobenzidine, and 3,3'-dimethylbenzidine. Higher amounts of benzidine are found in the urine of workers who perspire

freely and have a wet skin (Meigs, et al. 1954). Urinary benzidine measurements indicate that benzidine does not accumulate in body tissues, but no direct human tissue determinations have been performed to absolutely establish this concept (Meigs, et al. 1951).

PHARMACOKINETICS

Absorption and Distribution

Benzidine is rapidly absorbed into rats after intravenous injection. Maximum concentrations of free and bound benzidine are found at two and three hours, respectively. The highest concentrations were found in the blood, followed by liver, kidney, spleen, heart, and lung (Soloimskaya, 1968). Body distribution of benzidine in various tissues and urine 4 and 12 hours after intraperitoneal injection of 100 mg/kg was as follows: high concentrations in the stomach, stomach contents, and small intestine at 4 hours; and in the small intestine and its contents at 12 hours (Baker and Deighton, 1953). The amine content of the erythrocytes was low at both time intervals. Conjugated material, indicative of metabolites, was high in tissues and urine at 12 hours. Benzidine concentrations in the liver, the target organ for toxicity in rats, were relatively high and constant over the 12-hour period. When rats were given 20 mg of 3,3'-dimethylbenzidine subcutaneously once a week for eight weeks, the highest amine content was found in the Zymbal's gland followed by the kidney, omentum, spleen, and liver (Pliss and Zabezhinsky, 1970).

Metabolism and Excretion

A pharmacokinetic study of benzidine uniformly labeled with ^{14}C and dichlorobenzidine labeled in the 3,3' positions indicated

that substitution in the 3,3' positions of the benzidine molecule significantly affects the routes of metabolism and excretion. The blood half-life for benzidine was 68 hours in the rat and 88 hours in the dog. The weekly excretions of a dose of 0.2 mg/kg of benzidine in the rat, dog, and monkey were 97, 96, and 83 percent respectively. The excretion values for the dichloro compound were 98, 97, and 88.5 percent, respectively. Biliary excretion appears to be the main route of excretion of the dichloro compound in all three species. The dog and monkey excrete free benzidine with the urine, while the rat uses the biliary route. The urinary bladder of the dog had a high content of benzidine, suggesting that this is the reason for urinary bladder cancer in this species (Kellner, et al. 1973).

The various metabolites reported for benzidine and its congeners are given in Table 1. It can be seen that various species handle these chemicals in different ways and that the animal metabolites differ considerably from those excreted by humans. The improvements in analytical techniques have made identification of differences more positive. Of greatest interest are the human studies which will now be discussed.

A single oral dose of 100 mg of benzidine to a human resulted in the excretion of free benzidine and its mono- and diacetylated conversion products in the urine. The entire dose was not recovered indicating that fecal excretion probably occurred. This cannot be proven because the feces were not analyzed (Engelbertz and Babel, 1953). People ingesting 200 mg of benzidine excreted free benzidine and N-hydroxyacetylamino benzidine in their urine

TABLE 1

Metabolites Formed by Biotransformation of Benzidine and Benzidine Derivatives in Animals

Compound	Species	Metabolites	Reference
Benzidine	Human	Acetyl N-hydroxy compound	Troll, et al. 1963
	Human	N-Hydroxy acetylaminobenzidine	Haley, 1975
	Human	Monoacetylbenzidine and diacetylbenzidine	Haley, 1975
	Human	3-Hydroxybenzidine	Haley, 1975
	Human	3,3'-Dihydroxybenzidine	Haley, 1975
	Monkey	Monoacetylbenzidine	Rinde and Troll, 1975
	Dog	3-Hydroxybenzidine and glucuronide	Troll and Nelson, 1958
	Dog	3-Hydroxybenzidine hydrogen sulfate	Sciarini and Meigs, 1958
	Dog	3-Hydroxybenzidine	Bradshaw and Clayson, 1955 Sciarini, 1957
	Dog	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Dog	4-Amino-4-hydroxybiphenyl	Haley,1975
	Dog	Monoacetylbenzidine and diacetylbendizine	Haley, 1975
	Guinea pig	4'-Acetamido-4-aminodiphenyl	Clayson, et al. 1959
	Guinea pig	4'-Acetamido-4-amino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Guinea pig	4'-Amino-4-diphenyl sulfamic acid	Clayson, et al. 1959
	Guinea pig	N-Glucuronides	Clayson, et al. 1959
	Guinea pig	4'-Acetamido-4-diphenyl sulfamic acid	Clayson, et al. 1959

TABLE 1 (Continued)

Compound	Species	Metabolites	Reference
Benzidine	Rabbit	3-Hydroxybenzidine sulfate and glucuronide	Troll and Nelson, 1958
	Rabbit	4'-Acetamido-4-aminodiphenyl	Clayson, et al. 1959
	Rabbit	3-Hydroxybenzidine	Clayson, et al. 1959
	Rabbit	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Rabbit	4'-Amino-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rabbit	4'-Acetamido-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rabbit	N-Glucuronides	Clayson, et al. 1959
	Rat	3,3'-Dihydroxybenzidine	Haley, 1975
	Rat	N-Glucuronides	Elson, et al. 1958 Clayson, et al. 1959
	Rat	4'-Acetamido-4-Aminodiphenyl	Clayson, et al. 1959
	Rat	3-Hydroxybenzidine	Clayson, et al. 1959
	Rat	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Rat	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Rat	4'-Amino-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Rat	4'-Acetamido-4-diphenylyl sulfamic acid	Clayson, et al. 1959
	Mouse	Monoacetylbenzidine and diacetylbenzidine	Sciarini and Meigs, 1961a
	Mouse	Monoacetylated 3-hydroxybenzidine glucuronide and/or ethereal sulfate	Sciarini and Meigs, 1961a

TABLE 1 (Continued)

Compound	Species	Metabolites	Reference
Benzidine	Mouse	N-Hydrogen sulfate and/or glucuronide	Sciarini and Meigs, 1961a
	Mouse	3-Hydroxybenzidine glucuronide	Sciarini and Meigs, 1961a
	Mouse	4'-Acetamido-4-amino-diphenyl	Clayson, et al. 1959
	Mouse	4,4'-Diamino-3-diphenyl hydrogen sulfate	Clayson, et al. 1959
	Mouse	4'-Acetamido-4-amino-3-diphenylyl hydrogen sulfate	Clayson, et al. 1959
	Mouse	N-Glucuronides	Clayson, et al. 1959
3,3'-dimethyl-benzidine (orthotolidine)	Human	Diacetyl-o-tolidine	Dieteren, 1966
	Human	5-Hydroxy-o-tolidine	Dieteren, 1966
	Human	Monoacetyl-o-tolidine	Dieteren, 1966
	Dog	5-Ethereal sulfate of o-tolidine	Sciarini and Meigs, 1961b
3,3'-Dimethoxy-benzidine (dianisidine)	Dog	Unidentified diamine metabolite	Sciarini and Meigs, 1961b
3-Methoxybenzidine (monosubstituted dianisidine)	Rat	4-Amino-4'-acetamido-3-methoxybiphenyl	Laham, 1971

(Troll, et al. 1963). In the urine of plant workers exposed to benzidine in unknown quantities, free benzidine, its mono- and diacetylated derivatives, and 3-hydroxybenzidine were identified. The latter compound comprised 78.5 to 89.7 percent of the total (Sciarini and Meigs, 1961a). This work was a repeat of an earlier study by Meigs, et al. (1954) and confirmed the previous findings. It has been suggested that an 8-hour exposure to an air concentration of 0.018 mg/m^3 of benzidine would result in a urinary excretion of not more than 0.026 mg/l of diamines. Thus, an air exposure to 0.02 mg/m^3 or less of benzidine would be safe (Meigs, et al. 1954).

Dyestuff factory workers exposed to benzidine excreted free benzidine, 4-amino-4-oxybiphenyl, and monoacetylbenzidine in their urine (Vigliani and Barsotti, 1962).

Exposure to 3,3'-dimethylbenzidine results in urinary excretion of free 3,3'-dimethylbenzidine, its diacetyl derivative, and 5-hydroxy-3,3'-dimethylbenzidine. Although the monoacetylated derivative was not detected, there is a probability of its formation because 3,3'-dimethylbenzidine appears to be metabolized similarly to benzidine (Dieteren, 1966).

3,3'-Dichlorobenzidine has been identified in the urine of workers handling benzidine yellow. This establishes the weakness of the azo linkage in dyes made from this compound (Akiyama, 1970).

It is questionable how comparable animal data are to human data, and whether the former allow predictions to be made concerning the metabolic conversion of chemicals in various species. This is taken into consideration in the following discussion of the

animal data in Table 1 and their relevance to the human situation. Intraperitoneal injection of 100 mg/kg of benzidine in mice produced free benzidine, mono- and diacetylated derivatives as well as the ethereal sulfates and glucuronates of 3-hydroxybenzidine (Sciarini and Meigs, 1961a). The same dose of benzidine in dogs caused the excretion of free benzidine and conjugates of 3-hydroxybenzidine but no acetylated derivatives, because the dog lacks this biotransformation mechanism (Sciarini, 1957). The ethereal sulfate of 3-hydroxy-benzidine has been identified in dog urine and constitutes 25 to 50 percent of the administered dose (Sciarini and Meigs, 1958). The ethereal sulfate and glucuronide were the only metabolites found in dogs given 1 g of benzidine or rabbits given 100 to 300 mg of this chemical (Troll and Nelson, 1958).

The differences in the biotransformation of benzidine by the rat, mouse, rabbit, guinea pig, and dog are related to the presence or absence of specific enzymatic pathways. For example, the dog cannot acetylate benzidine. The rat, rabbit, and guinea pig can produce 4'-amino- and 4'-acetamido-4-diphenylyl sulfamic acid whereas the mouse and dog cannot. Other metabolites found were 4'-acetamido-4-aminodiphenyl, 3-hydroxybenzidine, 4,4'-diamino-3-diphenylyl hydrogen sulfate, and 4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate in the rat; and 4'-acetamido-4-aminodiphenyl, 4,4'-diamino-3-diphenyl hydrogen sulfate, and 4'-acetamido-4-amino-3-diphenylyl hydrogen sulfate in the mouse. 4,4'-Diamino-3-diphenylyl hydrogen sulfate was absent from rabbit and guinea pig urine, although the other metabolites were present. 3-Hydroxybenzidine and 4,4'-diamino-3-diphenylyl hydrogen sulfate were present

in dog urine. In all cases, N-glucuronides were present (Clayson, et al. 1959). Metabolite differences occur when different routes of elimination are considered. Dogs excrete the same benzidine metabolites in urine and bile but their feces have no 3-hydroxybenzidine or N-glucuronides (Clayson, et al. 1959). Comparison of routes of excretion of benzidine and its dichloro derivative in rats, dogs, and monkeys showed that the rat eliminated both compounds in greater quantities in the feces than in the urine, whereas the dog eliminated the dichloro compound to a greater extent in the feces. Neither route was decisive in the monkey, but more of both compounds did appear in the urine (Kellner, et al. 1973). Previously, it had been shown that dog fecal excretion of dichlorobenzidine was 10 times greater than urinary excretion (Sciarini and Meigs, 1961b), while the opposite was true for benzidine (Sciarini and Meigs, 1958).

When benzidine-based azo dyes were fed to monkeys, benzidine and monoacetylbenzidine were found in their urine (Rinde and Troll, 1975). This shows that the monkey, like man, can reductively cleave the azo linkage (Akiyama, 1970).

Intraperitoneal injection of dimethylbenzidine, dimethoxybenzidine, and dichlorobenzidine in dogs resulted in recovery of part of these chemicals in nonmetabolized form. The dichloro compound was not metabolized, whereas the other two derivatives of benzidine were recovered from urine as unidentified conjugated ethereal sulfates (Sciarini and Meigs, 1961b).

EFFECTS

Acute, Subacute, and Chronic Toxicity

In vitro studies have shown that benzidine, 3,3'-dimethylbenzidine, and 3,3'-dimethoxybenzidine are moderate reducers of cytochrome c. 3,3'-Diaminobenzidine is a strong reducer, whereas 3,3'-dichlorobenzidine is an ineffective reducer. It has been suggested that there is a relationship between carcinogenic potential and the reduction of cytochrome c (Hirai and Yasuhira, 1972; Cammer and Moore, 1973).

There is a significant increase in urinary B-glucuronidase activity in workers exposed to benzidine. The elevated activity, although decreased by removal from benzidine exposure, does not return to normal levels (Kleinbauer, et al. 1969; Popler, et al. 1964).

While 3,3'-dimethylbenzidine administered subcutaneously to rabbits had no effect on blood phenolase activity, benzidine decreased the activity of this enzyme (Nakajima, 1955). Rats injected with benzidine showed reduced catalase and peroxidase activity as well as a reduction in erythrocytes and thrombocytes and an increase in leucocytes (Soloimskaya, 1968). An intraperitoneal dose of 12.7 mg/kg of benzidine in rats increased liver glutathione from 182 mg/100 g to 272 mg/100 g in 24 hours (Neish, 1967).

Dermatitis has been reported in workers in the benzidine dyestuff industry, involving both benzidine and its dimethyl derivative. Individual sensitivity plays a prominent role in this condition (Schwartz, et al. 1947).

Glomerulonephritis and nephrotic syndrome have been produced in Sprague-Dawley rats fed 0.043 percent N,N'-diacetylbenzidine.

Both sexes developed proteinuria in 3 to 4 weeks. After two months the females were excreting 0.1 g of protein per 24 hours. The females developed severe anemia, which was rarely seen in the males. The former also had a hypoproteinemia, hyperlipemia, and generalized edema. Glomerular lesions in the females consisted of florid epithelial crescents, progressive sclerosis, and glomerular obliteration. In the males, the lesions were slower in developing and less extensive, but all males showing the nephrotic syndrome also developed testicular atrophy. There were morphological similarities between the human nephrotic syndrome and that induced by N,N'-diacetylbenzidine in rats, including extracapillary cell proliferation, formation of luxuriant crescents in 80 percent of the glomeruli, intact glomerular tufts, and the presence of normal glomeruli in the advanced stages of the syndrome (Harman, et al. 1952; Harman, 1971).

Rats fed N,N'-diacetylbenzidine or 4,4,4',4'-tetramethylbenzidine developed glomerular lesions with fat-filled spaces in the glomerular tuft from 2 to 4.5 months of treatment (Dunn, et al. 1956). Severe glomerulonephritis developed in rats receiving N,N'-diacetylbenzidine by subcutaneous (100 mg) or intraperitoneal (100 or 200 mg) injections. These lesions were dose-related (Bremner and Tange, 1966). A similar low grade glomerulonephritis has been produced in rats fed benzidine (Christopher and Jairam, 1970).

Mice fed 0.01 and 0.08 percent benzidine dihydrochloride developed the following toxic symptoms: decreased carcass, liver, and kidney weights; increased spleen and thymus weights; cloudy swelling of the liver; vacuolar degeneration of the renal tubules; and hyperplasia of the myeloid elements in the bone marrow and of

the lymphoid cells in the spleen and thymic cortex. There was a dose-dependent body weight loss of 20 percent in males and 7 percent in females. Moreover, male mice were more sensitive to benzidine than female mice (Rao, et al. 1971). This disagrees with Harman's (1971) findings in rats, but it may only be a species difference in response.

Synergism and/or Antagonism

Pertinent data could not be located in the available literature.

Teratogenicity

Embryonic mouse kidney cultures have an increased survival time but show hyperplastic epithelial changes in the presence of 3,3'-dimethylbenzidine (Golub, 1969; Shabad, et al. 1972). Administration of 8 to 10 mg of 3,3'-dimethylbenzidine to mice during the last week of pregnancy resulted in lung adenomas and mammary gland tumors in their progeny. These tumors could have resulted from transplacental transmission of the chemical or from its presence in the milk (Golub, et al. 1974). No teratogenic effects of benzidine derivatives in humans have been reported.

Mutagenicity

The results of the Ames assay on the mutagenicity of benzidine are positive (Ames, et al. 1973; McCann, et al. 1975; Garner, et al. 1975). With metabolic activation, benzidine causes an increase in the recovery of histidine revertants in Salmonella typhimurium strain TA 1537 and TA 1538, both sensitive to frameshift mutagens. The greatest increase was seen with TA 1538.

Another more recently developed assay, used to screen for putative mutagenic/carcinogenic compounds, has been used to test benzidine. This assay detects the inhibition of DNA synthesis by test compounds in HeLa cells (Painter and Howard, 1978). The concentration of a compound that is required to inhibit DNA synthesis by 40 percent corresponds with its mutagenic effects in Salmonella typhimurium. Benzidine has been shown to be positive in this DNA synthesis inhibition test (Painter and Howard, 1978).

Results of a Salmonella mutagenesis assay indicate that benzidine causes a significant increase in the reversion index of tester strains TA 98 and TA 1538 when the compound is activated by the addition of human liver microsomes (U.S. EPA, 1978).

Carcinogenicity

Benzidine and its derivatives are carcinogenic in both experimental animals and humans. In the latter these chemicals have been shown to produce bladder cancer after a long period of latency (Clayson, 1976). Additionally, these compounds produce dermatitis, cystitis, and hematuria in humans, indicating an early attack on the urinary bladder and presenting a sign that unless exposure is stopped, cancer may result (Haley, 1975). Table 2 gives various animal species and the type of cancer induced in them by benzidine and its congeners. It should be noted that only the dog develops urinary bladder cancer similar to that seen in humans after exposure to benzidine. The animal cancers, in general, differ significantly in their locations. This may be related to differences in specific target tissues or to differences in excretory pathways.

TABLE 2

Effects of Benzidine, Its Congeners, and Metabolites
On Various Animal Species*

Species	Carcinogen	Effect
Mouse	Benzidine	Hepatoma, lymphoma, bile duct proliferation
	3,3'-Dihydroxybenzidine	Hepatoma, lymphoma, bile duct proliferation, benign bladder papilloma
Rat	Benzidine and its sulfate	Cirrhosis of liver, hepatomas, carcinoma of Zymbal's gland, adenomacarcinoma, degeneration of bile ducts, sarcoma, mammary gland carcinoma
	3,3'-Dihydroxybenzidine	Hepatoma, adenocarcinoma of colon, carcinoma of forestomach, Zymbal's gland carcinoma, bladder carcinoma
	Dianisidine[a]	Zymbal's gland carcinoma, ovarian tumor
	o-Ditoluidine[b]	Papilloma of stomach, Zymbal's gland carcinoma, mammary tumor, leukemia
	3,3'-Benzidinedioxyacetic acid	Papilloma of bladder, hepatic sarcoma
	3,3'-Dichlorobenzidine	Extensive cancer
	N,N'-Diacetylbenzidine	Chronic glomerulonephritis
Hamster	Benzidine	Hepatoma, liver carcinoma, cholangiomas
	o-Ditoluidine[b]	Bladder cancer
Rabbit	Benzidine	Proteinuria, hematuria, liver cirrhosis, myocardial atrophy, bladder tumor, gall bladder tumor
Dog	Benzidine	Recurrent cystitis, bladder tumor, convulsions, liver cirrhosis, hematuria
Monkey	Benzidine	No pathological changes (Duration of study too short)
Human	Benzidine	Bladder tumor, papilloma, chronic cystitis, hematuria

*Source: Haley, 1975

[a] 3,3'-Dimethoxybenzidine.

[b] 3,3'-Dimethylbenzidine.

In some cases, excessive dosage may cause death due to toxicity, thus preventing the development of bladder cancer (Haley, 1975).

Benzidine and many other aromatic amines attack the urinary bladder and other organs (Hueper, 1954). However, it is the metabolites of these compounds that are considered to be the proximate carcinogens (Clayson, 1969). These aromatic amines are ring hydroxylated, converted to N-hydroxylated, acylated and deacylated derivatives, and conjugated with sulfate and glucuronide (Haley, 1975). It has been suggested that the conjugated N-hydroxy compounds are the active carcinogens in vivo. Bladder cancer has been induced in rabbits and dogs fed benzidine, but these findings are controversial (Haley, 1975). Spitz, et al. (1950) induced papillary carcinoma in 1 of 7 dogs fed benzidine for five years, but the cancer only appeared 7.5 years after the beginning of the experiment. Orally administered benzidine did not produce urinary bladder cancer in dogs (Marhold, et al. 1967). No tumors were found in female beagle dogs fed 1 mg/kg five days per week for three years (Deichmann, et al. 1965). In these last two studies, the lack of a carcinogenic effect in dogs is probably related to the known, long latency for benzidine cancer induction and the shortness of both studies.

Extensive bile duct proliferations and cysts appeared along with cholangiofibrosis, hepatomas, and liver cell carcinoma, but no urinary bladder tumors were found in hamsters fed benzidine at 0.1 percent of the diet throughout their life spans (Saffiotti, et al. 1967).

Benzidine administered subcutaneously to rats at a rate of 15 mg/week produced liver injury, cirrhosis, hepatomas, sebaceous gland carcinomas, and adenocarcinomas of the rectum, but no bladder tumors (Spitz, et al. 1950). Rats fed 0.125 percent of dihydroxybenzidine in the diet developed liver cirrhosis, hepatomas, adenocarcinomas of the colon, Zymbal's gland carcinoma, and squamous cell carcinomas of the stomach. One sessile papilloma and two keratinized squamous cell carcinomas were found in the bladder wall (Baker, 1953). Intraperitoneal or subcutaneous injection of N,N'-diacetylbenzidine in Wistar rats induced tumors of Zymbal's gland and of the mammary glands 6 to 15 months later. Glomerulonephritis was also reported and appeared to be dose-related. Female Sprague-Dawley rats given oral doses of 12 to 50 mg/rat developed mammary gland carcinomas (Griswold, et al. 1968).

Early cirrhosis occurred in rats given benzidine by subcutaneous injection for six months (Pliss, 1963). Injection site sarcomas, hepatomas, and Zymbal's gland tumors were also found and constituted 70 percent of the tumors in these rats (Pliss, 1964). Benzidine was more toxic to the females. Tumors of Zymbal's gland and the liver were induced by 3,3'-benzidine-dicarboxylic acid within one year (Pliss, 1969). Benzidine, in 5 mg weekly doses, produced intestinal tumors in rats (Pliss, et al. 1973). A cumulative dose of 0.75 mg/kg of benzidine for 15 days produced tumors in 20 of 22 rats, including 19 hepatomas, 18 cholangiomas, 7 intestinal tumors, and 4 sebaceous gland carcinomas. Subcutaneous tetramethylbenzidine doses of from 4.15 to 8.3 g/kg produced benign tumors at the injection site (Holland, et al. 1974).

Female Wistar rats given a single intraperitoneal injection of 100 or 200 mg of N,N'-diacetylbenzidine subcutaneously developed Zymbal's gland and mammary gland tumors after 6 to 15 months. The 100 mg intraperitoneal injection produced tumors in 11 out of 18 rats while the 200 mg dose gave no tumors (Bremner and Tange, 1966).

Hepatomas, bile duct proliferation, and benign papillomas of the urinary bladder were found in Delph albino mice injected subcutaneously with 300 mg of benzidine or dihydroxybenzidine. Only the latter chemical caused the bladder changes (Baker, 1950).

Benzidine or 3,3-dihydroxybenzidine administered subcutaneously at 6 mg weekly for 52 weeks produced tumors in exposed mice in 70 weeks. Benzidine induced hepatomas and lymphomas while the 3,3-dihydroxy derivative induced lymphomas and benign intestinal polyps. The significance of the lymphomas is obscure because one-third of the controls developed this condition spontaneously (Bonser, et al. 1956). Subcutaneous administration of 3,3-dihydroxybenzidine in mice caused tumors of the liver and mammary glands as well as leucosis (Pliss, 1961). Inner organ tumors developed after skin application of the chemical. Subcutaneous weekly doses of 6 mg of benzidine to C3HA mice induced hepatomas in 31 of 46 animals after 15 to 16 months. One animal developed a pulmonary adenocarcinoma (Prokofjeva, 1971).

3,3-Dimethylbenzidine in a cumulative dose of 5.4 g/kg for 241 days induced 11 gastrointestinal tract tumors, 7 hepatomas, 7 bone tumors, and 4 Zymbal's gland carcinomas in rats. Total oral doses of 500 mg in Sprague-Dawley rats produced 4 mammary carcinomas in 9

months in 3 of 16 surviving animals (Griswold, et al. 1968). Subcutaneous injection of 3,3'-dimethylbenzidine in rats caused skin tumors, large sebaceous gland tumors, and mammary tumors in 60 to 70 percent of the animals. When 20 mg of the chemical was implanted subcutaneously, hepatocellular carcinomas and subcutaneous sarcomas were produced (Pliss and Zabezhinsky, 1970).

3,3'-Dimethoxybenzidine given subcutaneously to rats induced Zymbal's gland tumors in two animals and an ovarian tumor and a fibroadenoma of the mammary gland in another one (Pliss, 1963). Both male and female Fischer strain rats developed tumors of the gastrointestinal tract, skin, breast, and ear duct after receiving 260 oral 10 mg doses of 3,3'-dimethoxybenzidine. The period of latency was 293 days (Weisburger, et al. 1967).

Subcutaneous administration of 3,3'-dichlorobenzidine to rats induced tumors in 74 percent of the animals (Pliss, 1963). Tumors appeared in the skin, sebaceous and mammary glands, intestines, bones, and urinary bladder. Dichlorobenzidine given by ingestion or injection into the underlying fat produced sarcomas at the injection site, an adenocarcinoma in the intestine, papillomas in the urinary bladder, and tumors in the sebaceous and mammary glands (Pliss, 1959). Total doses of 300 mg/rat orally of dichlorobenzidine produced no tumors (Griswold, et al. 1968). Rats fed 1,000 mg/kg in the diet developed mammary gland tumors in both sexes and Zymbal's gland and hematopoietic tumors in males (Stula, et al. 1971, Stula, et al. 1975). Progeny of BALB/c mice given total subcutaneous doses of 8 to 10 mg of dichlorobenzidine had a significant increase in tumor incidence. Tumors developed in 13 of

24 mice, with 4 adenocarcinomas of the mammary gland, 5 lung adenomas, and 7 cases of lymphatic leukemia (Golub, et al. 1974).

The carcinogencity risk for workers exposed to benzidine is 14 times higher than for the unexposed population (Case, et al. 1954). In the American dyestuff industry, 24 cases of bladder carcinomas were found in workers exposed to aromatic amines, including benzidine. The latency for tumor development was 12 years (Gehrman, 1936). In England the tumor induction time averaged 16 years, but one case occurred in two years (Case, et al. 1954). In 30 cases of bladder tumors the induction period varied from 8 to 32 years, with an average of 15.9 years. The concentration of benzidine in the exposure appeared to be the main factor in early tumor induction. Benzidine manufacturing was associated with 14 papillomas, 7 carcinomas, and two cases in which the papillomas were converted to carcinomas (Scott, 1952). Only a few weeks of exposure followed by a latent period of several years can produce bladder tumors (Deichmann and Gerarde, 1969). A latent period of 18.6 years has also been reported (Hamblin, 1963). Initial exposure concentration, exposure duration, and years of survival following exposure as well as work habits and personal hygiene are involved in the development of carcinomas where benzidine appears to be implicated (Rye, et al. 1970). There is little doubt that benzidine exposure is associated with an increase in the occurrence of bladder cancer (International Agency for Research on Cancer (IARC), 1972; Riches, 1972; Sax, 1975). However, there is a lack of information on the exact concentrations of benzidine to which workers have been exposed.

Long exposure to benzidine produced bladder tumors in 13 out of 25 men (Zavon, et al. 1973). Comparison of the two groups showed that the tumor group was exposed to benzidine for an average of 13 years while the nontumor group was exposed for an average of less than 9 years. Observations were carried out for approximately 12 years following exposure. Ambient air benzidine in the plant varied from 0.005 to 0.415 mg/m^3 with one area giving a value of 17.6 mg/m^3 (Wendel, et al. 1974). Death records of 171 workers showed that 18 were due to bladder and kidney cancers and that there was a higher rate of neoplasms of the digestive system. It appeared that there could have been a synergistic effect between benzidine and β-naphthylamine, since these workers were exposed to both chemicals (Mancuso and El-Attar, 1966, 1967).

When benzidine dyestuff manufacturing begins in any country the incidence of bladder tumors among exposed workers increases. Table 3 shows the times of discovery of aromatic amine bladder cancer in a number of countries. Urinary system tumors occurred in 17 percent of the workers in one benzidine plant. The highest rate of tumors was in the group exposed for 6 to 10 years (Kuzelova, et al. 1969). Men working in a French aromatic amine plant developed bladder tumors. One Normandy factory had 54 cases, with 17 occurring prior to 1947 and 34 subsequent to 1947. Symptoms of hematuria and stranguria were found in 18 cases (Billiard-Duchesne, 1960).

In Italy, 24 cancers were found in workers exposed to benzidine or benzidine-β-naphthylamine (Vigliani and Barsotti, 1962). Italian benzidine workers were found to have developed 47 cases of bladder cancer during the period from 1931 to 1960. There

TABLE 3

Time of Discovery of Aromatic Amine Bladder Cancer by Country*

Country	Year
Germany	1895
Switzerland	1905
United Kingdom	1918
U.S.S.R.	1926
United States	1931
Austria	1932
Italy	1936
Japan	1940
France	1946

*Source: Haley, 1975

were 21 carcinomas and 16 papillomas. During the period from 1931 to 1948, 13 of 83 workers developed bladder carcinomas from benzidine (Barsotti and Vigliani, 1952). The greatest exposure occurred in workers in filtration, pressing, drying, and milling of benzidine. Maximum latency for benzidine tumors was 16 years from the cessation of exposure. Ten papillomas and seven carcinomas were found in a cohort of 858 benzidine dyestuff workers (Forni, et al. 1972).

Studies in dyestuff plants in Japan showed 100 cases of bladder cancer during the period 1949 to 1970. Benzidine production workers accounted for 11.25 percent of the cases and benzidine users for 1.45 percent. Eight cases developed cancer of the upper urinary tract and not the bladder. There was a long latent period of 16.25 years (Tsuchiya, et al. 1975). The silk kimono painters are the highest risk bladder cancer group in Japan because they point their brushes, thereby ingesting benzidine dyes (Yoshida and Miyakawa, 1973).

There was a high incidence of bladder tumors (21.3 percent) in benzidine workers in a coal tar dye factory. The latent period was 18.4 years for papillomas and 18.7 for carcinomas (Goldwater, et al. 1965). A further study showed that the combined exposure to benzidine plus β-naphthylamine increased the bladder cancer rate to 45.5 percent (Kleinfeld, et al. 1966). Occupational bladder cancers are morphologically similar to spontaneous bladder tumors found in the general population. Both have a tendency for high recurrence after treatment.

At the present time there is no evidence that 3,3'-dimethylbenzidine, 3,3'-dimethyoxybenzidine, or 3,3'-dichlorobenzidine are human bladder carcinogens (Rye, et al. 1970). However, future epidemiological study may show them to be carcinogenic agents. No bladder neoplasms related to exposure to 3,3'-dichlorobenzidine over a 35-year period were found. However, the following neoplasms were reported in 17 workers: 2 lung cancers, 1 bone marrow cancer, 6 lipomas, 3 rectal papillomas, 2 sigmoid colon carcinomas, 1 prostate carcinoma, 1 breast muscle myoblastoma, and 1 basal cell epithelioma (Gerarde and Gerarde, 1974). No bladder tumors were found in British workers handling this chemical but the worker exposure time of less than 16 years could account for these findings (MacIntyre, 1975). It is possible that the latent period for bladder tumors is longer for 3,3'-dichlorobenzidine, since workers exposed to benzidine plus dichlorobenzidine developed such tumors, while those exposed to the latter compound alone did not (Gadian, 1975).

CRITERION FORMULATION

Existing Guidelines and Standards

In 1973 the U.S. EPA proposed, but did not promulgate, a toxic pollutant standard for benzidine (30 FR 35388).

The industrial standards instituted by the Occupational Safety and Health Administration (OSHA) in 1974 excluded from regulation any compounds containing less than 0.1 percent benzidine. These standards did not recognize a safe level of water contamination and provided no provisions for environmental monitoring.

New standards for benzidine discharges have been proposed (41 FR 27012) based upon information on the toxicological and environmental effects and the fate of benzidine. These standards, promulgated in 1977, established an ambient water criterion for benzidine of 0.1 µg/l. Effluent standards were set at 10 µg/l (daily average) with a maximum for any single day of 50 µg/l. Based on a monthly average, daily loading was limited to 0.13 kg/1000 kg of benzidine produced. The standards set for users of benzidine-based dyes were the same except that the maximum daily effluent concentration of benzidine was limited to 25 µg/l (42 FR 2617).

Current Levels of Exposure

It is essential that consideration be given to the manner in which benzidine and its congeners and the dyes derived from them contaminate water supplies. In most cases these chemicals are a hazard only in the vicinity of dye and pigment plants where wastes escape or are discharged. A field survey of the Buffalo and Niagara River areas using the chloramine-T method, with a

sensitivity of 0.2 µg/l, showed no benzidine in the samples. However, this method of analysis is photosensitive and leads to low estimates of benzidine. Moreover, the samples may have been below the level of detectability, or oxidative degradation may have converted the benzidine compounds to materials not detectable by the analytical method used (Howard and Saxena, 1976). A Japanese survey of the Sumida River area detected 0.082, 0.140, and 0.233 mg/l of benzidine in the water. The authors believed that the benzidine came from azo dyes by H_2S or SO_2 reduction (Takemura, et al. 1965).

Information on 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dichlorobenzidine and their dye derivatives as water contaminants is nonexistent, and research should be instituted to correct this deficiency.

It has been stated that benzidine resists physical and biological degradation (Lutin, et al. 1965; Malaney, et al. 1967; Radding, et al. 1975). Benzidine in water is oxidatively degraded by free radical, enzymatic, or photochemical processes (Radding, et al. 1975). Its half-life in water has been estimated to be 100 days. Air oxidation of benzidine in water seems to occur readily (Howard and Saxena, 1976).

Humic material seems to bind 3,3'-dichlorobenzidine tightly and its degradation appears to be slower than benzidine, but the half-lives of the two compounds are the same (Radding, et al. 1975). There is no information available on the dimethyl and dimethoxy derivatives. This deficiency must be corrected.

Benzidine is converted to a chloramine type compound during water chlorination processes (Jenkins and Baird, 1975). Soil and

intestinal bacteria reduce benzidine azo dyes to free benzidine (Yoshida and Miyakawa, 1973), and although aquatic organisms might also cause this same transformation, no data are available to prove this point. It should be remembered that the hydrochlorides of benzidine are much more soluble in water than the free amines and are more resistant to degradation than the latter (Bowman, et al. 1976).

Special Groups at Risk

A potential health hazard exists in the production of benzidine and its congeners and their conversion to azo dyes. There is no maximum permissible level of contamination in the industrial environment, although there are specific regulations governing the manufacture of benzidine and its congeners (39 FR 3756). These standards have reduced the risks to benzidine workers.

The use of benzidine and its congeners poses a potential risk to workers in biochemical, chemical, and microbiological laboratories where these chemicals are used as analytical reagents (Collier, 1974; Veys, 1972; Wood and Spencer, 1972). The greatest risk occurs in laboratories working with known carcinogens when good laboratory practices are not enforced. No epidemiological evidence is available to determine the exact extent of the problem.

The risk to the general population from benzidine, its congeners, and their dyes is unknown, but contamination of water supplies, which is known to occur in Japan (Takemura, et al. 1965), poses a yet to be determined risk. There also is a potential risk for workers in the garment, leather, and homecraft industries where the benzidine dyes are used.

Basis and Derivation of Criteria

The available data concerning the carcinogenicity of benzidine in experimental animals are severely limited. It is extremely difficult to extrapolate the experimental results to man because, with the possible exception of the dog and the rabbit, the target organs are different. Moreover, the metabolites produced by the various species, in general, differ significantly from those produced by man (Haley, 1975), although 3-hydroxybenzidine and its conjugation products are common to both man and animals.

Despite the limitations of the available data, a suggested criterion for benzidine was calculated using a relative risk model described in the appendix. The calculation assumes a risk of 1 in 100,000 of developing cancer as a result of daily consumption of 2 liters of benzidine-contaminated water and the daily consumption of 6.5 g of benzidine-contaminated aquatic organisms. Based on the data of Zavon, et al. (1973), a benzidine criterion of 1.2×10^{-3} µg/l is suggested to be adequate to protect the population consuming the water.

Epidemiological data indicate that exposure to benzidine is associated with an increase in bladder cancer in man. The possibility that benzidine may be found in wastewater may also pose a problem. In order to determine the extent of the potential problem, measurements must be made of wastewater, not only for benzidine, but also for its congeners. Moreover, further evaluation must be made on these chemicals and their azo dye derivatives to determine their stability to microbiological degradation. It is essential that studies of their carcinogenicity in experimental animals be

made at doses which produce a bare minimum of liver pathology. A detailed pharmacokinetic study should be undertaken to establish routes of absorption, body transport, storage, and excretion of benzidine, its congeners, and the azo dyes synthetized from them. Programs covering both industrial hygienic and epidemiologic aspects of exposure to benzidine and its congeners to establish the degree of dermal and pulmonary absorption are a necessity if we are to prevent this chemically induced cancer from occurring.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, -ero) consistent with the protection of aquatic organisms, human health, and recreational activities." Benzidine is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of benzidine in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of benzidine corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, the U.S. EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6} or 10^{-7} as shown in the table below.

Exposure Assumptions (daily intake)	Risk Levels and Corresponding Criteria(1)		
	10^{-7}	10^{-6}	10^{-5}
2 l of drinking water and consumption of 6.5 g of fish and shellfish (2)	1.2×10^{-5} µg/l	1.2×10^{-4} µg/l	1.2×10^{-3} µg/l
Consumption of fish and shellfish only.	5.3×10^{-5} µg/l	5.3×10^{-4} µg/l	5.3×10^{-3} µg/l

(1) Calculated from the relative risk model for epidemiology studies as described in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document and in Appendix 1. Appropriate data used in the calculation of the model are also presented in Appendix I. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000, and so forth.

(2) Approximately 22 percent of benzidine exposure results from the consumption of aquatic organisms which exhibit an average

bioconcentration potential of 87.5-fold. The remaining 78 percent of benzidine exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of benzidine, (1) occurring from the consumption of both drinking water and aquatic life grown in water containing the corresponding benzidine concentrations and, (2) occurring solely from the consumption of aquatic life grown in the waters containing the corresponding benzidine concentrations.

Although total exposure information for benzidine is discussed and an estimate of the contributions from other sources of exposure can be made, this data will not be factored into the ambient water quality criteria formulation because of the tenuous estimates. The criteria presented, therefore, assume an incremental risk from ambient water exposure only.

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APPENDIX I

Summary and Conclusions Regarding the Carcinogenicity of Benzidine*

Benzidine ((1,1'-biphenyl)-4,4'-diamine) is used in the manufacture of dyes, as a reagent for detection of H_2O_2 in milk, and as a reagent for hemoglobin.

It appears that the greatest hazard to exposure from benzidine occurs during its manufacture. Absorption through the skin is the primary route of entry into the body, although other routes of exposure such as inhalation and ingestion also exist. Exposure to benzidine, its derivatives, and other chemicals involved in the manufacture of dyes has long been known to be associated with an elevated incidence of bladder cancer in workers in Germany, England, Italy, France, Switzerland, Japan, and the United States (see Haley, et al. 1975, Zavon, et al. 1973, Clayson, 1976).

Epidemiological data clearly demonstrate that benzidine is a bladder carcinogen in humans, and experimental evidence indicates that it can induce cancer in a variety of organs in several species of animals. Several animal studies have reported carcinogenic effects of benzidine in hamsters (liver), rats (liver and Zymbal's gland), and mice (liver). Dogs have been reported to develop urinary bladder tumors following chronic exposure to large doses of benzidine (Spitz, et al. 1950; Bonser, et al. 1956). However, the

*This summary has been prepared and approved by the Carcinogens Assessment Group, EPA, on July 15, 1979.

small numbers of animals involved make the significance of these findings questionable.

The difference in organotropic properties of benzidine among the different species is probably due to both its route of excretion and metabolism. For example, in humans and dogs, benzidine or its metabolites are largely excreted through the urine, whereas in mice and rats, excretion is largely through the bile. In man, 70 to 90 percent of benzidine is excreted in the urine in the form of 3-hydroxybenzidine. In rats, it is questionable whether this metabolite is even formed, but it is formed in the dog and rabbit.

Three studies have reported mutagenic activity of benzidine towards Salmonella typhimurium (TA 1537 and TA 1538) in the presence of a rat liver mixed function oxidase system (Ames, et al. 1973; McCann, et al. 1975).

The carcinogenic and mutagenic activities of benzidine in animal systems clearly substantiate the epidemiological findings that show benzidine to be carcinogenic in humans. In a recent report, The National Academy of Sciences (NAS, 1975) calculated an estimate of the total benzidine exposure of occupationally exposed humans on the basis of the urinary levels. The NAS report presented a table comparing tumor incidence and total accumulated dose in humans and two species of laboratory animals.

Table 1 contains data from the NAS (1975) report as well as additional animal data. On the basis of the data presented in this table, it is apparent that in animal studies where benzidine was injected and where liver tumors were induced, much higher doses of benzidine were required than in the sensitive mammary tumor rat

TABLE 1

Degree of Exposure and Reported Cancer Frequencies for Agents Carcinogenic to Man and Laboratory Animals

	Conditions of Exposure	Total Accumulated dose (mg/kg)	Cancer	Reference
Man	11.46 yr; occupational	130	52% bladder (13/25)	Zavon, et al. 1973
Mouse	1/wk; 32 - 52 wks S.C. injection	10,000	67% liver (31/46)	Prokofjevea, 1971
Mouse	1/wk; 52 wks S.C. injection	10,400	12% liver (7/60)	Bonser, et al. 1956
Rat	1/wk; 64 wks S.C. injection	3,200	4% liver (6/152)	Spitz, et al. 1950
Rat*	1/3 days for 30 days gastric intubation	100 50 control	78% mammary (7/9) 50% mammary (5/10) 4% mammary (5/132)	Griswold, et al. 1968

*dose calculated on basis of an average rat weight of .25 kg, from NAS, 1975.

model system and in the doses estimated to give a high bladder cancer incidence in man.

The data from the human epidemiology study of Zavon, et al. 1973 was used to estimate the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5}. In this study 25 workers in a benzidine manufacture plant, were observed for the appearance of bladder cancer over a period of 13 years. In this series 13 of 25 men developed bladder tumors after a mean exposure period of 13.61 years, their average age at the end of exposure was 44 years and at the end of a 13 year observation was 57 years. The men not showing evidence of cancer had a mean exposure period of 8.91 years, their average age at the end of exposure was 43 years and at the end of observation 56 years. The estimated total accumulated dose of 130 mg/kg was estimated from average urinary levels of benzidine in these workers at the end of a workshift (see Table 1 and Zavon, et al. 1973). From this data the concentration of benzidine in water calculated to keep lifetime cancer risk below 10^{-5} is 1.2×10^{-3} µg/l.

Four animal studies shown in Table 1 were considered for possible use in the calculation of the water quality criterion. The most sensitive response occurred in the Griswold study, where 10 to 20 female Sprague-Dawley rats per treatment group were administered benzidine by gastric intubation in ten equal doses at three-day intervals over a 30-day period and observed for nine months. Total doses of 25 and 12 mg/l benzidine/rat induced carcinomas in 7/9 and 5/10 animals, respectively, compared to 5/132 animals in the control group. All tumor-bearing rats had multiple carcinomas and one

had a fibroadenoma. Based on these data, the concentration of benzidine in water, calculated to keep the lifetime cancer risk below 10^{-5}, is 8.5×10^{-4} µg/l.

Although the criterion value derived from human exposure data is higher than that calculated from the most sensitive animal system, it seems reasonable that human epidemiological data are most appropriate for estimating human risks. The study of Zavon, et al. (1973) was selected as the data base for deriving the water quality criterion. Based on these data, the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5} is 1.2×10^{-3} µg/l.

Summary of Pertinent Data

The data from the human epidemiology study of Zavon, et al. (1973) was used to estimate the concentration of benzidine in water calculated to keep the lifetime cancer risk below 10^{-5}. In this study 25 workers in a benzidine manufacturing plant were observed for the appearance of bladder tumors after a mean exposure period of 13.61 years, their average age at the end of exposure was 44 years and at the end of a 13 year observation was 57 years. The men not showing evidence of cancer had a mean exposure period of 8.91 years, their average age at the end of exposure was 43 years and at the end of observation 56 years. The estimated total accumulated dose of 130 mg/kg was estimated from average urinary levels of benzidine in these workers at the end of a workshift (see Table 1 and Zavon, et al. 1973). The criterion was calculated from the following parameters:

Average weight of man = 70 kg

Observed incidence of bladder cancer = 13/25 (52 percent)

Bioconcentration factor of benzidine = 87.5

X = average daily exposure producing lifetime risk of 10^{-5}

B* = potency factor, which is an estimate of the linear dependency of cancer rates on lifetime average dose

C = concentration of benzidine in water, calculated to produce a lifetime risk of 10^{-5}, assuming a daily ingestion of 2 liters of water and 0.0065 kg fish.

The average duration of benzidine exposure for this cohort was 11.46 years. The average urine level of benzidine at the end of a workshift was approximately 0.04 mg/l of urine. Assuming the average urine output per day for a man is 1.2 l/day the expected urine concentration would be

$$0.04 \text{ mg/l} \times 1.2 \text{ l/day}$$

$$= 0.0480 \text{ mg/day worked}$$

Assuming a recovery factor of 1.45% (i.e., 1.45% of the actual exposure concentration was found in the urine according to Rhinde and Troll, 1974) and assuming an average human body weight of 70 kg, the estimated exposure is

$$\frac{0.0480}{0.0145} \div 70 = 0.0473 \text{ mg/kg/day worked}$$

Assuming that an average of 240 days are worked in a year, the resulting average lifetime exposure would be

$$0.0473 \times \frac{240}{365} \times \frac{11.46}{56.5} = 0.0063 \text{ mg/kg/day},$$

where 11.46 is the average duration of exposure and 56.5 years is the average age of the cohort at the end of study.

The carcinogneic potency of benzidine is estimated, using the model

$P = 1 - \exp\left[-Bdt^3\right]$, as

$$B = \left[-\ln(1 - 13/25)\right] \div \left[0.0063 \times (56.5/71.3)^3\right]$$

$$= 234.13 \text{ (mg/kg/day)}^{-1},$$

where 71.3 years represents the average life span in the U.S. and 56.5 years is the average age of the cohort at the end of the study.

Therefore, the water concentration is calculated as:

$$C = \frac{70 \times 10^{-5}}{234.13\ (2 + 0.0065 \times 87.5)}$$

$$= 1.16 \times 10^{-6} \text{ mg/l}$$

$$= 1.16 \text{ ng/l.}$$